THE INHERITANCE OF BLUE MERLE

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Blue merle is a complex character, far more than just a colour. The old description that it is a dominant gene, with deleterious effects when homozygous, is sufficient to trace the character's inheritance in pedigrees. Additional recent knowledge paints a more complex and interesting picture. Unfortunately, misunderstanding of some of these complexities has resulted in a series of negative myths about blue merle.

Blue merle results in the "washing out" of black and some black-related brown pigments to a bluish gray. One of the most desirable blue colours is pale enough to resemble cigarette smoke (smoke blue in the rest of this article), but from there the colour may be darker and darker until it can be described as steel blue or darker. I have seen two puppies, sired by a smoke blue merle dog, on which the merle mottling was so dark that, from the other side of a show ring, I was baffled by the colour. I had to approach them to assure myself that they were truly blue. In addition, on many, but not all, blue merles, the blue darkens as the dog ages, so that smoke blue veterans are uncommon.

All blue merles have black patches that are unpredictable in size and placement. While most blue merles show the characteristic bluish gray, with irregular black patches, that is highly variable. At one extreme, the dog is smoke blue with white trim and a few small black spots, while at the other, there is so little blue that it may be missed, or blanked out by white. Cryptic merles have little or no blue, and therefore little evidence of the blue and irregular black pattern. Where a small patch of blue is covered by white, it will be entirely masked, as white conceals all the colours which underlie a white patch. The key feature of such dogs is that they will produce merle offspring when bred to a normal tricolour. One Cardigan special in the USA in the late 1970s had a pattern similar to a reticulated giraffe: there were palm-sized black patches surrounded by borders of blue less than a half inch wide.

The blue merle may have brown eyes, pale blue eyes, or eyes with brown and blue patches. A blue merle Cardigan lacks a layer called the tapetum in the retina, such that, in the dark, the eyes of blue merles reflect red, where the eyes of normal dogs reflect green. There are other conditions that result in a defective tapetum and reflect red, but so far I have never encountered a merle having green reflection. My canine ophthalmologist tells me that merles with brown irises show some green in the eye when examined closely, and may give a yellowish reflection. However, on the dog of mine that she used to show this, (one brown eye, one blue) both reflected red as I drove up the driveway in the dark. The red reflection allows tentative identification of merle carriers which show no merling in the coat, although a few dogs will thus be called merle when they are not. As noted below, there will soon be a genetic test for the merle condition.

The white or china eye as seen in Siberian Huskies also occurs in Cardigans, but is totally unrelated to the merle gene. These blue eyes also reflect red in the dark. Brown eyes related to that condition sometimes reflect red, sometimes green, whereas the brown eyes of blue merles always reflect red, in my limited experience. I must emphasize that the red reflection is a rough and ready test for blue merles, but not 100% accurate.

Normal blue merle Cardigans are heterozygous for the merle gene, having the genotype **Mm**. We know this because normal breedings are blue to tri (**Mm x mm**), and, in the long run, puppies are evenly divided between blues and blacks. I use shorthand designations to describe phenotypes, that is, the appearance of the dog.

Longhand description	Shorthand
Tricolour with tan points	tri-t
Black and white with brindle points	tri-br
Blue merle with tan points	blu-t
Blue merle with brindle points	blu-br

When a blue merle is bred to a sable, red or brindle, several unusual colours or patterns are possible. Unfortunately, due to the long-standing prohibition against actually doing such breedings, few of today's Cardigan fanciers have seen such dogs, with the result that the names of the colours are so sloppily applied that one cannot tell what the colour actually was. The two most common such terms are ginger merle (blue x brindle) and peach merle (blue x sable or red). A brindle x merle cross may appear to be brindle, but instead of regular dark stripes, the dark will be in irregular patches, rather like the black on a normal blue merle. There may be a bluish cast to the whole coat, hard to describe, but obvious when you see it. Just to be thoroughly confusing, I have seen a litter which contained a red tricolour dilute ("Dudley") and two of its blue equivalent, which were blues with cinnamon-coloured spots where the black should have been, and a cinnamon cast over the blue. So, should we call these cinnamon merles? I would rather not use such terms at all, because they are so confusing.

Collie and Sheltie breeders commonly breed blues to sables. The sable merles have the red or tan undercoat, and where the dark overlay would be, it may appear in irregular patches, or be lacking. I have examined a sable merle Smooth Collie where the only evidence of merling was on the backs of the ears.

The tri pattern comes with many variations. The black may be deep blue-black, normal shoe-polish black, or somewhat sooty. The points on the face may range from two small tan spots on the lower cheeks, with slightly larger than match-head sized spots over the eyes all the way to the very open red pattern of the sable's "monk's cap", where the eye spots are larger and wrap around the eyes, to be separated from the tan of the cheeks only by the black line which goes from outer corner of the eye to the edge of the ear (and occurs in both sables and tri-t). On top of the head, and on the backs of the ears, the undercoat may be a rich tan or red, and, since the guard hairs in those locations are short, the red will show through. At least two fully red-headed tri-t Cardigans have been shown in the USA. A blu-t is nothing more than a tri-t with most of the black turned to blue. That will not, repeat not, change the distribution of the tan on the points. So lots of tan trim on the face, head and ears (as well as legs and under tail) is a perfectly normal occurrence for blu-t. I have responded to too many panicked inquiries from people whose blue puppy has a reddish head, and they fear it is the dreaded ginger (actually peach?) merle!!

Tri-br dogs have much more black on the points, so blu-br dogs tend to have only a little bit of brownish wash on the cheeks, etc. The stripes of brindle do not show clearly on most tri-br or blu-br dogs. If you imagine one of those tri-br dogs that only has a dozen brown hairs on each cheek, you can see where a blu-br might show no obvious brown on the points at all.

A further complication arose in 2005, when it was finally confirmed that **e e** reds occur, if only very infrequently, in Cardigan Corgis. Actually, I personally experienced this in 1976, when I bred a tri-br male to a blu-t female. We would normally expect to see only blue merle and black puppies in such a litter, but to my surprise there were also two pale red puppies, out of a total of seven! The other five were normal blues and tris. At that time, no one had a supportable explanation of the phenomenon. The recent confirmation of e e in the breed started from a breeding that was almost identical - a tri-t x blu- breeding produced two red puppies. Then, a tri x tri breeding produced red puppies, something that had never before been reported in the breed. Dr. Sheila Schmutz analysed DNA from these puppies, and confirmed that they were **e e** reds. The **e e** genotype is a totally different source of reddish colour from the more familiar a^y a^y genotype. A Cardigan which has the **e e** genotype and also has the Mm genotype will not show any evidence of merling in the coat. However, it will still have red reflection from the eyes.

Homozygous merles have several variations. First, let's consolidate terminology. Many people call a homozygous merle a double merle. I will distinguish the two merles by designating the homozygous as **MM**-blu, and the heterozygous will be, as above, **Mm**-blu, or simply blu, with a suffix for the points colour.

The merle gene is said to be partially lethal when homozygous. If you breed a blu-t dog to a blu-t bitch, you would expect that a quarter of the puppies would be MM-blu, half would be blu-t, and a quarter would be tri-t. In fact, fewer than 10% of puppies from a blu x blu cross are **MM**-blu merles. That probably indicates that more than half such puppies die in utero and are resorbed. Those that are born have some or all of the following characteristics:

1. They are mostly white, typically with only a few spots of colour 1 - 2 inches in diameter.

2. They are very likely totally deaf from birth.

3. They may have very small eyes, or the irises may be defective, giving a star-like pattern to the pupil. Those with small eyes may be blind or partially blind.

4. In other breeds, many **MM**-blu puppies die within the first 10 weeks. That is not reported in Cardigans (among the few reports we have), but note that many Cardigan **MM**-blu seem to die in utero.

5. One of the first **MM**-blu dogs of any breed that I encountered personally was an Australian Shepherd which, the owner and I discovered, was not only deaf, but had lacked a sense of smell from birth. That is the only case of lack of smell that I have been able to find. Note that this dog was working open obedience entirely on hand signals, and doing very well. Clearly, despite being deaf and unable to smell, she was very intelligent and active.

MM-blu dogs are clearly defective in several respects. One of the persistent myths is that they will transmit these defects to all their puppies if they are bred. That is absolutely untrue!! Blu and tri puppies will be normal blues and tris!

The third major recent advance in Cardigan genetics is that there is now a solid explanation of the genetics of blue merle, albeit there are still some loose ends to be investigated. A paper entitled "Retrotransposon insertion in SILV is responsible for merle patterning of the domestic dog", written by Leigh Anne Clark, Jacquelyn M. Wahl, Christine Rees, and Keith E. Murphy, appeared in January 2006 in the *Proceedings of the National Academy of Sciences, Early Edition (on-line)*. This provides definitive evidence that merle is caused by a short interspersed element (SINE) inserted into the SILV locus at a known location. The average size of the insertion is 253 base pairs. SINEs are common in the dog genome, making up as much as 7% of the total genome.

A very rough analogy is that conventional genes are like recipes in a cookbook. SINEs are inserts into genes which are non-functional, and may disrupt the action of the gene. Think of the SINE as a passage of text from a machinists' manual on how to run a milling machine, and further that the computer has inserted the randomly cut piece backwards into your cookbook text. Worse, you are the "recipe reader" for a team of 100 chefs, and the meal has to be ready at a certain time. It is pretty clear that any non-functional text would interfere with your getting complex recipes done right and on time.

There have been historically several indications that blue merle was not a simple gene mutation. One important indicator was that the gene has multiple effects. It is semi-lethal when homozygous, and those few (less than half) homozygous merles that do survive are likely to be profoundly deaf from birth, and have a variety of eye problems.

A key piece of evidence was recognized in several other breeds, but only recently reported in Cardigans. That is that the reversion rate from merle to non-merle is much higher than normal gene mutation rates. If we call the merle allele **M**, and the allele for normal or non-merle **m**, the rate of reversion **M➜m** is in the order of 3 - 4%, whereas normal mutation rates are in the order of 1 in 10,000. A homozygous merle has the genotype **MM**. Bred only to tricolour (Tri-t or Tri-br) dogs, which all have the genotype **mm**, all the offspring should be **Mm**; in appearance these would all be normal blue merles. In breed after breed, it has been reported that homozygous merles bred to tricolors produce 3 – 4 % tricolour offspring (Clark et al.).

Before we can look in detail at reversion in Cardigans, we must remind ourselves of the quirks provided by the laws of chance. A key feature is to have enough observations. I would like to see at least 100, and preferably 500, puppies from MM x mm breedings before we start to talk about percentages with any expectation of precision. In 2004 Lore Bruder had orders for 3 blue merles. She bred a blue male to a tri bitch, and got 7 tris. The following February I had a litter from the litter brother of the sire Lore used (both are **Mm** blue merles), bred to a tri, and got 8 blues. Add these two up, and you have as close to 1/2 blue as you can get, but separately, well, we either won or lost the lottery. This is the same logic as every litter should have equal numbers of males and females. Most of us who have been at it a while have had 6 females, 0 males, then, a couple of years later 5 males and 0 females. The simplest way to calculate the probability of such events to use the formula for getting runs of heads and tails when you flip a coin. That results in the following numbers:

The chance of getting *p* Blues to *q* Tris in a normal Blue x Tri breeding are, expressed in the chance of this happening out of 1000 litters (that's right, a thousand tries!!).

8	blues,	0	tris	= 3
7	blues,	1	tris	= 31
6	blues,	2	tris	= 109
5	blues,	3	tris	= 219
4	blues,	4	tris	= 273
3	blues,	5	tris	= 219
2	blues,	6	tris	= 109
1	blue,	7	tris	= 31
0	blue,	8	tris	= 3

Betty Ann Seeley has provided an estimate of the reversion rate of the **M** gene in Cardigans. Her Pecan Valley Double Exposure (call name Kodak) bred always to **mm** tris has sired 46 puppies, of which 3 were tris. That seems outside the 3-4% range suggested by the Clark et al. paper, but it's close, and the small sample size demands that we allow "wiggle room". However, 3 out of 46 is very different from 23 out of 46 tris, which is what you would expect if Kodak were, as some have suggested, a bizarrely white-splashed normal blue, that is, of genotype **Mm** instead of **MM**. So I believe Kodak is a homozygous merle (**MM**), and that his three tri offspring are evidence that blue is a transposon rather than a simple gene. That also agrees with what is reported in Australian Shepherds, Shelties, Collies and Great Danes.

If sire A (**MM**) were mated to Dam B (**mm**) enough times to accumulate 100 puppies, the result should be 100 out of 100 blue merle puppies, if **M** were a normal mutant gene. The fact that it was 96 or 97 out of 100 blues (Clark et al.), or 94 if Kodak has provided an honest estimate of reversion in Cardigans, is clear evidence that we are not dealing with a conventional gene. If the breeding were **Mm** x **mm**, we would expect to get 50 blues to 50 tris, but, in fact, we would get about 47 blues to 53 tris. Statistically, you would need a lot more than 100 puppies to show that 50:50 is different from 47:53. So no wonder we can only detect reversion by breeding double merle (**MM**) to tri (**mm**). If merle were simply a dominant gene, there should only be one tri out of 10,000. (I am assuming that the approximate 1/10,000 mutation rate would apply.)

This sort of insertion of a blocking or non-functional piece of genetic material into a normal gene is far from unique to blue merles. Consider the following quotations from the Clark et al. paper:

> [1] "Characterization of SILV in merle and nonmerle Shetland Sheepdogs revealed a short interspersed element (SINE) insertion at the intron10/exon11 boundary. The SINE segregates with the merle phenotype in multiple breeds and is absent from dogs representing breeds that do not have merle patterning."
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> [2] "PCR was carried out by using genomic DNA from two nonmerle, one blue merle, and one double merle Shetland sheepdog to obtain amplicons from each exon of SILV. Amplification of exon 11 yielded two products: (i) the expected 206-bp <<base pair>> product and (ii) a larger product (slightly smaller than 500 bp). These amplicons segregated with the merle phenotype among the aforementioned dogs: The nonmerle dogs were homozygous for the 206-bp product; the blue merle was heterozygous for the products, and the double merle was homozygous for the larger product." The authors have promised a DNA based test for demonstrating the presence of the transposon, that is, the merle-causing element.
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> [3] "DNA was available from 50 of the 61 Shetland Sheepdogs used in the linkage analysis. These 50 dogs were analyzed by gel electrophoresis for the insertion. The insert was present in the heterozygous state in 12 merles and in the homozygous state in 2 double merles. Thirty-one nonmerle dogs did not harbor the insertion, and four nonmerle dogs were heterozygous for a smaller insertion. Sequence analysis of this smaller insertion from two Shetland Sheepdogs revealed a deletion within the oligo(dA)-rich tail of the SINE. This smaller insertion was also present in a nonmerle which is suspected to be cryptic because it was sired by a double merle; however, no test breedings have been conducted to date to conclusively classify the dog as cryptic."
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> [4] "A SINE, structurally similar to a class of canine SINEs described by Minnick et al (32), was identified in SILV for all merle dogs analyzed. This SINE shows high sequence similarity (95-97%) with canine SINEs previously identified in the canine D2 dopamine receptor gene (36), the dystrophin gene (37), and the PTPLA gene, implicated in centronuclear myopathy (38). These SINEs are t-RNA derived and highly abundant in the dog, representing 7% of the genome." The numbers in parentheses are references to papers in the literature cited. One of the surprises to me, when whole genomes and the genes that are part of those began to be sequenced, was how much non-functional (or unknown functioned??) DNA there is in the total genome. This last paragraph makers it sound as though the merle insertion, which we have called a mutation for many years, is a common event which had the bad (or should I say good?) luck to happen in a color gene. By the way, SILV is a color gene.

Quote [3] leads to a discussion of cryptic merles. First, we must be absolutely sure that we separate thoughts about phenotypic cryptic merles from genotypic cryptic merle.

A **phenotypic cryptic merle** is of normal genotype **Mm**, but shows little or no evidence of blue merle coloring. When bred, it will produce puppies as though it were a normally-colored blue merle. We are all familiar with the fact that some blue merles have few and small black spots, while other are more black than blue. Follow that last to an extreme, where the dog is almost entirely black. Even more extreme, imagine that the only patch of blue is overlain by white, so it can't be seen at all. That is a true phenotypic cryptic merle. Genetically it is a normal **Mm** blue merle, but by accident none of the blue shows through. This is the normal use of the term "cryptic merle" within the fancy.

There are two quite different types of **genetically cryptic blue merles**. The first occurs when expression of the **Mm** is blocked by the genotype **ee**. Such a red Cardigan would show no sign of being merle until it was bred. Please note also that not all **ee** reds are cryptic merles, as the **ee** condition can block brindle, sable and tricolour.

The second possible **genetically cryptic merle** is raised in quote [3] from Clark et al. They observed some normal non-merle Shelties that retained a shortened version of the transposon. If (a big if) that shorter piece can later regain enough length to make recipients merle, then this would be a cryptic merle. The case they mention is interesting, as the dog in question was a tricolour sired by a homozygous merle. I still think it is a myth that a homozygous merle can pass medical problems to its normal merle and normal tricolour offspring, but we certainly need to learn a lot more about these normally colored dogs that retain part of the transposon.

For the present I think we should be conservative, and consider all cryptic merles to be genetically normal merles, except for those ee reds found in a merle litter. Until we have more experience with tricolors which have a double merle as parent, I think it is premature to call any or all of them cryptic merles. One final point: any genetic change can occur in both directions, but the probability going the reverse direction is usually different from the probability of going in the forward direction. Reversion of the merle transposon is a good example of this. For the merle to be lost, all that is needed is for the transposed piece of chromosome to break off its unusual location. For blue merle to happen in the first place requires not only that a piece break off its normal site, but it also has to stick on to the new site. Thus, it is a much, much rarer event to get a blue merle from a tri x tri breeding than it is to get a tri from a homozygous merle x tri breeding. I have never heard of that happening, although, obviously, it did happen somewhere, some time, in some breed, or we would not have blues in the first place.

Dr. Murphy's team at Texas A&M have promised a commercial test for the merle condition, hopefully late in 2006. That will forever lay to rest the huge bugbear of former generations of Cardigan breeders, namely the fear that a dog could carry merle without showing it, such that a breeder could unknowingly breed merle to merle. The test will have similar power to the PRA test we have become familiar with. From a research point of view, it will help elucidate to nature of the reversion tricolors produced by **MM** x **mm** breedings.

As I thought about the reversion of the merle gene, an interesting idea occurred to me. Please note that it is no more than an educated guess. If the cause of the reversion occurs fairly frequently during the meiosis cell divisions which produce eggs and sperm, is it possible that it also reverts during the mitosis cell divisions which occur as a puppy progresses from a single fertilized cell to a complete dog made up of hundreds of millions of cells? If the merle condition were lost in places that would, in a future time, be the cells that produce hair, then the irregular black patches of the normal blue merle might be places where the merle condition was lost from an early cell that was ancestor to the patch. Thus, comparison of cells from merle and non-merle spots on the same dog may help us understand the mechanism which causes merle.

THE MYTHS AND MYSTIQUES OF BLUE MERLE

Breeding of blue merles to any colour other than tricolour (tri-t or tri-br) was banned in the USA in the late 1970s. Although there has been a great deal of debate, the reasons for the ban were never clearly elucidated. Partly as a result of that, a number of myths about the inheritance of blue merle have arisen. Some of these sound remarkably far-fetched, but all have come to me as questions about the inheritance of blue merle. I admit to being an expert if, and only if, the definition of expert is recognized to be "a drip, under pressure", as defined in Murphy's laws.

(1) The ostensible root of the ban was this: since the blue gene affects only black pigment, a red dog can carry the blue gene without showing it. There was the tragic possibility, then, that a novice could breed two red dogs with the resulting litter containing MM-blu puppies, deaf, perhaps blind, and with poor chances for survival. The realization that e e may be the underlying genes of one kind of red Cardigan makes this fear more real, because merling does not appear to show at all on such puppies. Such an outcome was considered sufficiently tragic that the breed club in the USA sought to minimize the probability that it would happen. With the knowledge of the present day, such an event seems unlikely. The lack of tapetum, resulting in red rather than green reflection from the eyes, is a useful clue. While that test is not 100% accurate, at least the errors are in the less harmful direction. That is, a very few non-merles will be called merles because their lack of tapetum, and therefore red reflection, come from conditions not related to merle at all. The china blue eye of Siberian Huskies, a condition also seen (infrequently) in Cardigans, results in red reflections. Actually, we should suspect that any Cardigan with blue, even small blue flecks, is a merle carrier, even though there is the "china" eye, totally unrelated to merle, in the breed. The red reflection is most useful when you are wondering whether a brown-eyed red dog might be carrying merle. Detection of inapparent carriers of the merle gene will soon (2006) be definitive, by a DNA test.

(2) There certainly is a legitimate ethical issue concerning blue to blue breeding. Is it right to carry out a breeding which is likely to produce one or more profoundly defective puppies? I believe that each individual must be free to ask and answer that question. The plus of blue x blue breeding is said to be that it results in paler, clearer blues, but there is no evidence in support of this theory.

(3) It is interesting to note in the context of off-colour merles that the colour blue merle had disappeared from the breed at the end of WWII. Thelma Gray of Rozavel recovered the colour by searching out red dogs that had blue flecks in their eyes and breeding these together. (http://www.cardicommentary.de/Coloursandcoats/merle.htm).

(4) I have frequently been asked if brownish colour or muddy blue colour was the result of there being one or more brindles in the pedigree. Once one recognizes that a blue merle is only a tricolour (tri-br or tri-t) with some of the black washed out, this possibility is eliminated. It is perfectly respectable to breed brindles such that tri-br or even tri-t puppies result from the mating, and such tris are within the normal range of tricolour shades and patterns. Thus, there are no bad features in having brindle dogs in the pedigree, as long as they were not mated directly to blue merles.

The panic which ensues when a blue x tri breeding results in a blu-t dog with a lot of tan on the face and head has been dealt with above. Again, this is to be expected, and is not a cause for concern.

(5) The role of the "Dudley" gene, similar to what produces the red tricolour in Dobermans, has been described. However, there is also a myth that this gene, far back in the pedigree, can produce off-colour merles. If, by "off-colour", one means muddy blue, or "too much" red

on the head, this is false. On the other hand, as this red or cinnamon colour is rare and recessive, it does turn up unexpectedly, and so in that case, known occurrence of a Dudley in the pedigree is a true predictor of a very occasional off-colour merle involving the „Dudley“ colour.

Dudleys in other colours than merle, on the other hand, are not caused by too many blue merles in the pedigree unless some of these were carriers of the Dudley gene.

(6) Merle is definitely dominant. That means that merle will not skip generations. Only if the merle colour is of small extent, or masked by white (cryptic merle), will merle ever be missed.

(7) As explained above, the health defects of **MM**-blu dogs are not passed down to **Mm** blu or tri offspring.

Merle is a colour to which very few people are neutral. Many Cardigan people admire the colour, others dislike it. It is interesting genetically, if you possess correct information and understanding. Most of the difficulties with the colour stem from poor understanding of the whole condition.

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